Amendments to the Claims

Claim 1-13 (canceled)

Claim 14 (currently amended) The method of claim ~~13~~ 25, wherein said analyte-selective reagent is colorimetric.

Claim 15 (canceled)

Claim 16 (currently amended) The method of claim ~~13~~ 25, wherein said reagent is renewed by a pump and at least one valve.

Claim 17 (original) The method of claim 16, wherein said pump and at least one valve are selected from the group consisting of at least one peristaltic pump, at least one syringe pump, at least one positive displacement pump, at least one solenoid pump and valve and at least one pinch valve.

Claim 18 (currently amended) The method of claim ~~13~~ 25, wherein said reagent is renewed by a solenoid pump and valve.

Claim 19 (canceled)

Claim 20 (currently amended) The method of claim ~~13~~ 25, wherein said reagent-based optical chemical sensor is a Submersible Autonomous Moored Instruments for CO_2.

Claim 21 (original) The method of claim 20, wherein said analyte-selective reagent is bromothymol blue.

Claim 22-24 (canceled)

Claim 25 (new) A method of operating absorbance-based chemical sensors to achieve calibration-free measurements, the method comprising the steps of:

a) establishing wavelength accuracy to within about 2-3 nanometers;

b) eliminating stray light at all wavelengths to about less than 0.1% incident light;

c) preparing an analyte-selective reagent at a concentration;

d) equilibrating the analyte-selective reagent to an analyte;

e) taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a first wavelength ($I_{\lambda 1}$) with a reagent-based optical chemical sensor, wherein the sensor has been modified to allow the renewal of an analyte-selective reagent, wherein the first wavelength corresponds to an un-reacted form of the analyte-selective reagent, and taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a second wavelength ($I_{\lambda 2}$), wherein the second wavelength corresponds to a reacted form of the analyte-selective reagent;

f) replacing the equilibrated analyte-selective reagent and analyte with a spectrophotometric blank solution;

g) taking an intensity reading of the blank solution at the first wavelength ($I_{\lambda 10}$), and taking an intensity reading of the blank solution at the second wavelength ($I_{\lambda 20}$);

h) calculating an absorbance ratio using the equation $A_R = A_{\lambda 1}/A_{\lambda 2}$, where A_R is the absorbance ratio, $A_{\lambda 1}$ is absorbance at the first wavelength and $A_{\lambda 2}$ is absorbance at the second wavelength and, wherein $A_{\lambda 1}$ and $A_{\lambda 2}$ are determined by

$$A_\lambda = -\log \frac{I_\lambda}{I_{\lambda o}}\text{; and}$$

i) calculating the sensor response with the molar absorptivities (ϵ) of the reacted (a) form of the analyte-selective reagent and the un-reacted form (b) of the analyte-selective reagent using the equation

$$R = -\log \frac{A_R - \epsilon_{\lambda 1a}/\epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b}/\varepsilon_{\lambda 2a} - A_R \epsilon_{\lambda 2b}/\epsilon_{\lambda 2a}} = +pK_a - pH.$$

wherein when the analyte-selective reagent is prepared accurately and reproducibly at the concentration sensor readings between sensors are calibration-free.

Claim 26 (new): The method of claim 21, wherein said first wavelength is 620 nanometers and said second wavelength is 434 nanometers.